

Sidestream Tobacco Smoke Exposure Acutely Alters Human Nasal Mucociliary Clearance

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Nasal mucociliary clearance (NMC) is a biomarker of nasal mucosal function. Tobacco smokers have been shown to have abnormal NMC, but the acute effect of environmental tobacco smoke (ETS) on nonsmokers is unknown. This study evaluated acute tobacco smoke-induced alterations in NMC in 12 healthy adults. Subjects were studied on 2 days, separated by at least 1 week. Subjects underwent a 60-min controlled exposure at rest to air or sidestream tobacco smoke (SS) (15 ppm CO) in a controlled environmental chamber. One hour after the exposure, ^{99m}Tc-sulfur colloid was aerosolized throughout the nasal passage and counts were measured with a scintillation detector. Six out of 12 subjects showed more rapid clearance after smoke exposure than after air exposure, and 3/12 had rapid clearance on both days. However, substantial decreases in clearance occurred in 3/12 subjects, all of whom had a history of ETS rhinitis. In two subjects, more than 90% of the tracer remained 1 hr after tracer administration (2 hr after smoke exposure). Understanding the basis for biologic variability in the acute effect of tobacco smoke on NMC may advance our understanding of pathogenesis of chronic effects of ETS. **Key words:** environmental tobacco smoke, inhalation toxicology, mucociliary clearance, nose, tobacco. *Environ Health Perspect* 103:1026–1030 (1995)

Environmental tobacco smoke (ETS) is one of the most common indoor pollutants and is associated with a range of adverse health effects in both children and adults (1–6). We have focused on acute irritant and rhinitis symptoms of variable magnitudes in healthy nonsmoking adults (7, 8) and are interested in understanding the mechanisms of increased upper respiratory infections and increased asthma symptoms among children (8).

Our previous studies characterized the acute physiologic and inflammatory response to sidestream smoke (7, 8). ETS is defined as the tobacco smoke that nonsmokers inhale; it is composed of sidestream tobacco smoke (SS) and exhaled mainstream smoke (MS). SS is the dominant component of ETS and can be generated with a smoking machine. We have examined the response to ETS by performing controlled human exposure studies with SS. Subjects with a history of ETS rhinitis symptoms demonstrate an increased symptomatic and objective congestive response to brief, high levels of SS (45 ppm for 15 min). Both subjects with and without a history of ETS rhinitis demonstrate a congestive response to prolonged moderate levels of SS (15 ppm for 2 hr). The congestive response lasts less than an hour and occurs without an increase in the permeability of the nasal vasculature, as indicated by no change in the concentration of albumin in the nasal lavage (7). Although the congestive response subsides within 1 hr after exposure, patients report symptoms lasting hours to days after exposure.

Clinically, a reduction in baseline mucociliary clearance indicates respiratory tract injury. Clearance of particles from the respiratory tract is an accepted biomarker of respiratory tract function response to toxicants (10), and clearance has been quantified using saccharine, charcoal, and radio-opaque or radiolabeled tracers (11). Proctor (11) concluded that a nasal mucociliary clearance (NMC) < 1 –2 mm/min was abnormal in healthy adults. Alterations in particle clearance have been used to characterize agent toxicity in animal studies (12). Twenty years ago, Anderson et al. (13) noted anecdotally that controlled SO₂ exposure caused the greatest inhibition of NMC in the human subjects who showed the greatest SO₂-induced nasal symptoms. These findings suggested the broad hypothesis that subjects reporting increased symptoms with exposure to an irritant might have inhibition of NMC. The purpose of the present study was to determine the effects of controlled sidestream tobacco smoke exposure on NMC in healthy nonsmokers.

Materials and Methods

Twelve healthy, nonsmoking subjects were recruited: six had a history of ETS sensitivity and an objective, congestive response to controlled challenge with SS, and six had no history of ETS sensitivity and no congestive response to controlled challenge with SS. The selection procedure was as follows: Healthy, nonsmoking, young adults (ages 18–45) were recruited by

advertising at the University of Maryland at Baltimore. "Nonsmoking" was defined as a lifetime cumulative smoking history of less than 1 pack-year and no smoking in the last 5 years. Subjects completed a health history questionnaire, and those with chronic cardiorespiratory conditions were excluded from the study. A screening questionnaire asked subjects to rate their history and severity of symptoms associated with exposure to environmental tobacco smoke on a 0–5 scale (0 = no history of symptoms, 1 = mild symptoms, 3 = moderate symptoms, 5 = severe symptoms). Symptoms of eye, nose, and throat irritation and headache that occurred with historical ETS exposure were summed to calculate a "historical ETS-irritation index" and symptoms of nasal congestion, rhinorrhea, sneezing, and postnasal drip that occurred with historical ETS exposure were summed to calculate a "historical ETS-rhinitis index." Subjects with an ETS-rhinitis index of ≤ 1 were termed "ETS nonsensitive (ETS-NS)," subjects with an index of ≥ 2 were termed "ETS sensitive (ETS-S)." This subject stratification has been used in prior studies at our research facility (7).

Prospective subjects underwent a screening challenge with SS (15 ppm CO, 1 hr) at rest. Those with a greater than 35% increase in nasal resistance were classified as demonstrating a congestive response to SS; those with a smaller than 5% increase in nasal resistance were considered nonresponsive, and those with an increase in nasal resistance between 5 and 35% were considered indeterminate. The 12 subjects were also characterized with a history, nasal inspection, baseline nasal and lung function, methacholine reactivity, and skin-prick test (7). Stratification was performed before controlled SS challenge followed by measurement of NMC. Subjects from the two groups were interspersed and data collection analysis and reduction completed before comparison of group differences.

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This research was supported by the Center for Indoor Air Research, Linthicum, Maryland. Received 17 April 1995; accepted 17 July 1995.

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The order of the study days was varied and study days were separated by at least 1 week. Figure 1 shows the study protocol.

An environmentally controlled research exposure chamber was used to limit exposure to the desired pollutant and to provide constant conditions of temperature ($22.2 \pm 0.5^\circ\text{C}$) and relative humidity ($40 \pm 2\%$). The environmental chamber air supply consisted of ambient air passed through high-efficiency particulate absolute (HEPA) filters to remove particles and activated carbon filters to remove gaseous pollutants. The environmental chamber consisted of a 41-m^3 clean room and a 22.2-m^3 exposure room. A ventilation rate of $5.0\text{ m}^3/\text{min}$ in the exposure room enabled a complete air change every 7.5 min. All air in the exposure room was exhausted to the outside without recirculation.

Reference cigarettes 2R1F (Tobacco and Health Institute, Lexington, Kentucky) were used for smoke generation, and SS was generated in a smoking chamber adjacent to the exposure room. The generation system consisted of a 4.4-ft^3 Plexiglas box containing three manifolds connected to a timing device. Intermittent puffs were generated by sequentially opening solenoid valves connected to each manifold to achieve a negative pressure puff on the filter end of the cigarettes. Human smoking patterns were simulated by using a 2-sec puff duration at a vacuum pressure of $13.7\text{ cm H}_2\text{O}$ to produce a 35-cc volume of draw on each cigarette at 1-min intervals. Mainstream smoke was exhausted to outside the building. Sidestream smoke was introduced into the exposure chamber through a ceiling diffuser and allowed to exit the chamber without recirculation. Burning cigarettes were replaced approximately every 10 min during the study. Levels of SS were characterized by carbon monoxide (CO) concentration in the exposure room, which was monitored continuously with an Ecolyzer Series 2100 CO Meter (Energetics Science, New York) and with a Bendix Model 8501-5CA Infrared Gas Analyzer (Bendix Instruments, Lewisburg, West Virginia). A target concentration of 15 ppm CO was achieved by controlling the number of cigarettes puffed. Subjects entered the exposure room after the target smoke concentration was reached. Particle concentrations and distribution from 0.3 to greater than $10\text{ }\mu\text{m}$ (Climet Model 226-210 Multi-channel Particle Analyzer, Climet Instruments Inc., Redlands, California) and total organic vapors (HNU-P1101, HNU, Inc., Charlestown, Massachusetts) were also measured during selected exposures. The organic vapor meter was calibrated daily

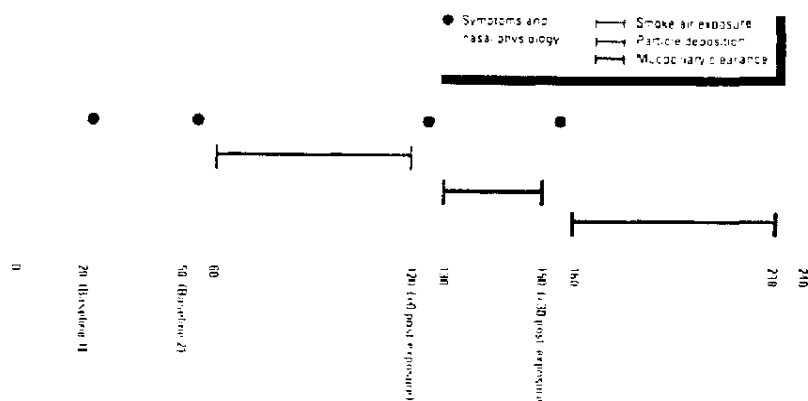


Figure 1. Protocol. Each subject was studied on 2 days, separated by at least 1 week, and exposed to clean air or sidestream tobacco smoke at a concentration of 15 ppm carbon monoxide. Nasal physiology measurements included symptoms measurements, posterior rhinomanometry and acoustic rhinometry and confirmed resolution of nasal congestion before aerosol delivery. The ^{99m}Tc -sulfur colloid aerosol was delivered 1 hr after completion of the smoke exposure.

with 100 ppm isobutylene. Clean-air exposures were conducted in an identical manner except that cigarettes were not burned.

We began to assess NMC 50 min after completion of smoke or air exposure, that time at which the acute nasal congestive response to smoke had resolved. An aerosol colloidal ^{99m}Tc -sulfur in normal saline (Sincor, Timonium, Maryland) was delivered to the nasal mucosa, with the catheter tip positioned 0.3 cm anterior to the inferior turbinate. A scintillation detector, placed anterior to the subject, made serial counts for 60 min at 2-min intervals for 30 sec each. Juxtaposing marks on the face and detector ensured consistent alignment. Values were normalized to the initial count and expressed as percent tracer remaining at each time point. Between 40 and 50 min after exposure, the activity remaining in each region of the nasal passage was measured using a collimated detector.

The conditions of aerosol administration were developed to effect diffuse deposition in the upper respiratory tract. Five additional subjects were recruited to examine the initial pattern of tracer deposition. The four additional detectors needed to make this assessment were not available at the time of the initial protocol, but these five subjects met the entry criteria for the initial part of the study. Subjects underwent measurements immediately after administration of the aerosol to the left nasal passage. The measurements were made with the detector in four positions: centered at 2.5 cm, 3.5 cm, 7.5 cm, and 10.5 cm posterior to the tip of the nose. Deposition was expressed as a percentage of the counts measured from the anterior position. In the five pilot subjects, the

activity measured by the anterior detectors was $26 \pm 9\%$ and deposition in the posterior two detectors was $13 \pm 11\%$ of the anterior detector counts.

Statistical analyses (ANOVA) were performed with Excel (Microsoft Corp., Redmond, Washington). Because the ETS-S and ETS-NS groups were selected and stratified in advance of the study, the effect of smoke on NMC was analyzed separately for each group.

Results

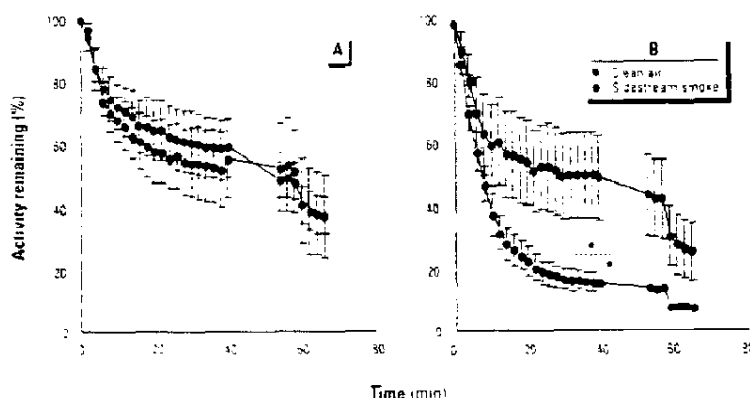
Table 1 shows the characteristics and nasal dimensions of subjects at the time of tracer administration (1 hour after air or smoke exposure).

Figure 2 shows the average NMC curves for the ETS-NS and ETS-S subjects. All subjects showed smooth decay curves, indicating consistent positioning and reproducible measurements. After clean-air exposure, the percent tracer remaining was similar in the two groups (ETS-NS $51 \pm 14\%$ versus ETS-S $41 \pm 10\%$, nonsignificant). After smoke exposure, the ETS-NS subjects showed accelerated NMC, with only $15 \pm 3\%$ tracer remaining (ETS-NS, air versus smoke, $p < 0.05$). In contrast, the ETS-S subjects had no change in mean clearance, with $46 \pm 13\%$ of the tracer remaining (ETS-S air versus smoke, $p = 0.86$).

Figure 3 shows the individual subject data 40 min after tracer administration. Six of 12 subjects showed accelerated clearance ($>35\%$ less tracer remaining after smoke exposure than after air exposure). Three of 12 fast-clearing subjects showed no change after smoke exposure compared with NMC on air-exposure days ($<30\%$ remaining on

Table 1. Nasal dimensions when radioactive aerosol was delivered to the nasal passage and activity remaining at 40 min after exposure^a

Subject no.	Characteristics ^b	Exposure	% Activity remaining	Area _{min} ^c	Volume (cm ³)	
					Anterior	Mid
ETS sensitive/SS responsive ^d						
1	F,26,NA	Air	65	1.1	3.5	32.3
		Smoke	26	0.7	2.0	12.5
2	M,23,NA	Air	84	0.7	2.5	27.2
		Smoke	49	0.7	2.2	22.0
3	F,26,A	Air	67	0.4	1.0	15.8
		Smoke	96	0.3	0.7	20.8
4	F,21,NA	Air	23	0.4	1.1	05.1
		Smoke	51	0.5	1.6	05.3
5	M,24,NA	Air	40	0.4	1.4	14.8
		Smoke	91	0.5	1.6	16.7
6	M,24,NA	Air	78	0.9	2.2	13.1
		Smoke	24	0.7	1.7	10.6
ETS nonsensitive, SS nonresponsive						
7	M,24,A	Air	25	0.5	1.3	15.2
		Smoke	21	0.5	1.6	17.3
8	F,27,A	Air	68	1.1	3.7	29.5
		Smoke	12	1.2	3.5	35.0
9	F,28,A	Air	87	0.4	1.0	08.7
		Smoke	14	0.5	1.3	14.8
10	F,25,A	Air	85	0.9	2.4	27.1
		Smoke	26	0.9	2.1	14.6
11	F,24,NA	Air	26	0.7	2.1	15.7
		Smoke	10	0.7	2.1	18.3
12	F,25,NA	Air	7	0.7	2.5	35.8
		Smoke	8	0.4	1.1	10.7

^aValues shown are the dimensions of the nasal passage in which the tracer aerosol was delivered.^bSex, age in years. A, atopic as determined by a positive wheal-and-flare response to one or more skin-prick tests; NA, nonatopic as determined by a negative response to skin-prick tests.^cMinimum nasal passage cross-sectional area.^dSensitive/responsive subjects reported a history of rhinitis symptoms with environmental tobacco smoke exposure (ETS sensitive) and demonstrated increased nasal resistance with a screening challenge with sidestream tobacco smoke (SS responsive).**Figure 2.** Total mucociliary clearance. (A) Results for the environmental tobacco smoke-sensitive (ETS-S) subjects ($n = 6$) and (B) results for nonsensitive (ETS-NS) subjects ($n = 6$). Clearance after air exposure and after smoke exposure are shown. Values are means \pm SEM of counts made at 2 min intervals for 30 sec per count. Clearance was normalized to the initial count. At 58 min an anterior nasal wipe was performed, demonstrating that on all days only a small quantity of the tracer was located in the nasal antrum. ANOVA, ETS-NS nasal mucociliary clearance (NMC) post-air vs. ETS-NS NMC post-smoke, $p < 0.005$, ETS-S NMC post-air vs. ETS-S NMC post-smoke, $p = 0.86$, NMC post-clean air ETS-S vs. ETS-NS, non-significant.

both days). Three of 12 subjects (all ETS-S) showed substantially increased retention ($>25\%$ more tracer remaining after smoke exposure than after air exposure). The three subjects showing reduced clearance with smoke compared to air exposure were subject nos. 3, 4, and 5. There was no significant correlation between the activity remaining at 40 min and the nasal dimensions at the time of aerosol administration.

Figure 4 shows the regional distribution of tracer 40 min after exposure in ETS-NS and ETS-S subjects. On average, ETS-S subjects showed increased retention of tracer at all sites in the nasal passage after smoke exposure.

Discussion

ETS is one of the most common indoor air pollutants, and understanding susceptibility to its adverse effects is of scientific interest and public health importance. The present data indicate that exposure to SS at levels similar to smoky, poorly ventilated rooms causes variable effects on NMC of healthy human subjects (14). The majority of the subjects demonstrated acceleration of NMC with SS exposure or maintained rapid clearance. However, in 3 of the 12 subjects, acute, controlled sidestream tobacco smoke exposure resulted in a substantial reduction in NMC.

The nasal mucociliary clearance system used in this study was designed to aerosolize the tracer throughout the nasal passage. This allows a search for either diffuse or focal effects within the nasal cavity. Animal toxicology studies show that mucosal lesions of the nasal passage differ in the site and type of lesion depending on the toxin (12). Other methods to assess mucociliary function, such as the saccharine, charcoal, and radio-opaque disc methods, only assess clearance in a single stream from the inferior turbinate to the pharynx (9).

A previous study examined the effect of exhaled mainstream smoke on nasal mucociliary clearance of nonsmokers (15). Ten healthy, nonsmoking volunteers smoked two cigarettes each, exhaling the smoke through their nostrils. There was no acute change in their nasal mucociliary function as detected by the mean ciliary beat frequency or mean nasal mucociliary clearance. Nasal mucociliary clearance was also studied in three donkeys exposed to tobacco smoke (8); no acute change in clearance occurred.

Mucociliary clearance is an aggregate measure of mucosal function and integrity (10). Determinants of normal function include the quantity and composition of airway surface fluid and the type and function of epithelial cells, especially ciliated

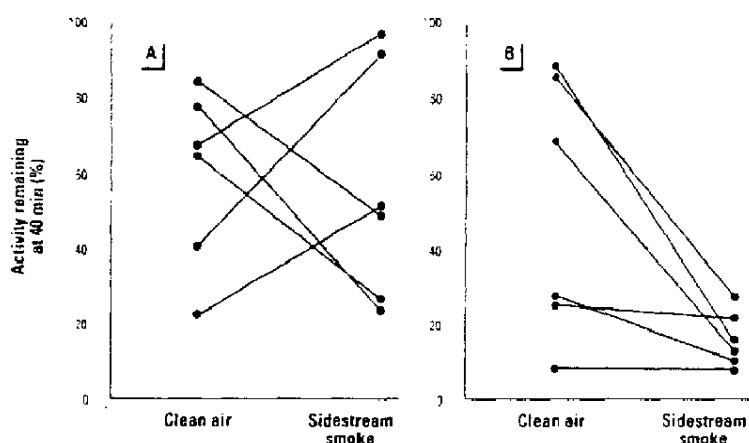


Figure 3. Activity remaining in the nasal passage 40 min after exposure. (A) Results for the environmental tobacco smoke-sensitive (ETS-S) subjects ($n = 6$) and (B) results for the nonsensitive (ETS-NS) subjects ($n = 6$).

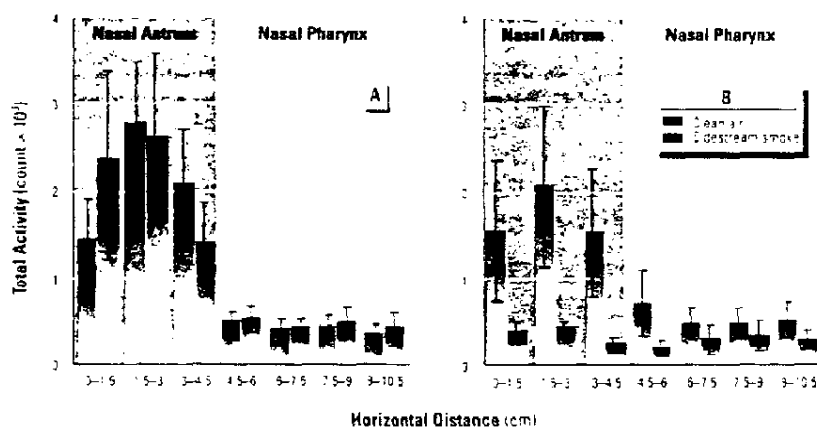


Figure 4. Distribution of activity remaining in the nasal passage. (A) Results for the environmental tobacco smoke-sensitive (ETS-S) subjects ($n = 6$) and (B) results for the environmental tobacco smoke nonsensitive (ETS-NS) subjects ($n = 6$). Total ETS-NS nasal mucociliary clearance (NMC) post-air vs. ETS-NS NMC post-smoke, $p = 0.06$. ETS-S NMC post-air vs. ETS-S NMC post-smoke, nonsignificant.

cells (12). Animal and *in vitro* studies indicate that airway cells may potentially be altered by cigarette smoke (12), although exposure levels used for animal studies were typically higher than those used in this study. In rodent models, tobacco smoke has been shown to cause acute increases in vascular permeability via activation of upper respiratory capsaicin-sensitive neurons, an effect that could increase airway surface fluid. However, previous human studies in our laboratory failed to show evidence for increased nasal vascular permeability with smoke exposure at these levels (7,10).

Studies of dogs have demonstrated an effect of stimulation of α -fiber neurons on ciliary beat frequency (17). These studies are relevant to the present study, as investigators have demonstrated that the acute response to tobacco smoke in the upper respiratory tract of rodents occurs via activation of capsaicin-sensitive, α -fiber nerves (18,19).

In the dogs, capsaicin, administered as an aerosol for 2 min, stimulates an increase in ciliary beat frequency. This effect is partially blocked by prior administration of indomethacin (a cyclooxygenase pathway inhibitor), ipratropium bromide (a muscarinic receptor antagonist), and hexamethonium bromide (a nicotinic receptor antagonist). The time course of the increase in ciliary beat was observed to include an early phase, lasting 15 min, and a subsequent phase, lasting 30 minutes (17). These data suggest that activation of the chemosensitive nerves with sustained increases in ciliary beat frequency could account for the increased mucociliary clearance observed in six of the subjects in this study.

The inhibition of NMC in three subjects after smoke exposure is of particular

interest. Nasal dimensions at the time of NMC measurement were similar on the 2 study days, making differences in initial tracer deposition unlikely. Small changes in NMC related to cyclic changes in nasal passage volume are also unlikely to account for the effects seen. Upper respiratory responses to numerous toxicants are quantitatively similar between humans and many experimental animals (10,20,21), and the mechanisms of mucociliary clearance are thought to be similar in humans and most other mammals. Animal studies have shown that low-level irritant exposure accelerates mucociliary clearance. Acceleration of NMC has been demonstrated in humans with exercise, attributed to adrenergic stimulation (nasal saline flush attributed to increased surface fluid), and pharmacologic agents such as β -agonists and cholinergic agonists.

Animal studies have shown that higher-level irritant exposure inhibits mucociliary clearance (2,22). Our data suggest a possible leftward shift in the exposure-response curve in some of our subjects (2), and mechanisms for the reduction in NMC include inhibition of ciliary beating, altered viscoelastic properties or reductions in airway surface fluid (23,24). In human studies, few stimuli other than extreme dehydration markedly inhibit NMC (11).

Other investigators have assessed the effects of active smoking on baseline nasal mucociliary clearance (7,9). A comparison of nonsmokers and smokers showed that 28 current smokers had a mean clearance time of 20.5 min, which was significantly longer than the mean time of 11.8 min in 27 lifelong nonsmokers. One smoker, not included in the average, had a clearance >60 min. There was no significant difference between the mean nasal ciliary beat frequency of 10 smokers and 10 nonsmokers (measured on nasal scrapings). The investigators interpreted this as evidence that the periciliary environment (e.g., surface fluid and mucus rheology) were responsible for the impaired clearance and that a direct ciliary toxicity was less likely. Studies of young smokers showed various degrees of impairment of tracheal mucous velocity before the onset of bronchitis symptoms or evidence of airway obstruction via pulmonary function tests (10,23).

The clinical significance of these findings remains to be determined. We speculate that the subjects with inhibition of nasal mucociliary clearance after smoke exposure are at increased risk of chronic airway injury for two reasons. First, mucociliary clearance is an accepted biomarker of pulmonary function (10), and

clearance is maintained in the face of many insults. Inhibition of clearance suggests a greater degree of injury than unaltered or accelerated clearance. Second, impaired mucociliary clearance has been associated with increased rates of infection and allergy, and these processes could produce further injury. Cystic fibrosis and ciliary dyskinesias (e.g., Kartagener's syndrome) are conditions characterized by impaired mucociliary clearance and increased infections. Animal studies have demonstrated that exposure to ozone inhibits pulmonary clearance of radiolabeled tracers and causes increased primary allergen sensitization and secondary anaphylaxis (24–26). Woodworkers with high dust-exposure levels had increased rates of impaired nasal clearance and higher infection rates than those with low dust-exposure levels (27).

Studies of the chronic alterations in mucociliary clearance in upper and lower respiratory tract disease have been interpreted as suggesting that loss of control of mucociliary transport could either cause or result from chronic respiratory disease (28). Adverse effects of active smoking include nonmalignant, chronic obstructive lung disease and upper and lower respiratory tract cancers (1,8,29). The basis for susceptibility to these effects is not well understood, even though numerous studies have shown familial aggregation of chronic obstructive lung disease (30,31).

Our test, coupling SS exposure with NMC, meets initial criteria for a biomarker of susceptibility for respiratory disease because there is a relationship to respiratory disease and because NMC is fairly sensitive to change after exposure to appropriate agents (10). Additional characteristics that would need to be determined to estimate the utility of NMC are its longitudinal stability and population distribution. Interpretation of these acute smoke-induced changes in mucociliary clearance in terms of potential health problems is speculative at present (10).

Understanding the mechanism of the variability in the NMC response to acute smoke exposure will help assess its relationship to the symptoms of ETS-rhinitis, assess whether it indicates subclinical mucosal injury in healthy subjects, determine whether there is an altered exposure-response curve, and determine if it is a marker of individual susceptibility to the chronic effects of mainstream or environmental tobacco smoke.

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